

EXHIBIT F

CONSUMPTION OF PRECURSORS OF N-NITROSO COMPOUNDS AND HUMAN GASTRIC CANCER

N.W. Choi¹, A.B. Miller², J.G. Fodor³, M. Jain², G.R. Howe⁴,
H.A. Risch², & A.M. Ruder¹

¹Department of Epidemiology and Biostatistics, Manitoba Cancer Treatment
and Research Foundation, and University of Manitoba, Winnipeg, Manitoba;

²NCIC Epidemiology Unit, Department of Preventive Medicine and Biostatistics,
University of Toronto, Toronto, Ontario; and ³Faculty of Medicine,
Memorial University of Newfoundland, St John's, Newfoundland, Canada

It has been hypothesized that dietary nitrate and nitrite are converted in the stomach to nitrous acid, which reacts with secondary amines and amides to form nitrosamines and nitrosamides, compounds frequently demonstrated to be carcinogenic in animals, and that vitamins C and E inhibit *N*-nitroso product formation by chemically reducing nitrous acid. This hypothesis and others were tested in a case-control study (controls were individually matched by age, sex and area of residence), utilizing a standardized, quantitative, dietary history questionnaire interview. Daily nutrient consumption values were calculated from interview responses, and continuous conditional logistic regression was used for the data analysis. Significant findings are as follows: (1) Average daily consumption of nitrite, chocolate and carbohydrate was associated with increasing trends in risk. (2) While citrus fruit intake appeared to be somewhat protective, any protective effect of vitamin C intake was less apparent, and of vitamin E, not at all apparent. (3) Consumption of dietary fibre was negatively associated with gastric cancer risk. These findings appear to implicate a number of dietary components, including nitrite consumption, in the genesis of gastric cancer in humans.

The incidence of and mortality from gastric cancer vary greatly from country to country, between migrating populations and their former compatriots, and between generations within some migrant racial groups. Numerous past studies of gastric cancer in migrant populations point to the importance of environmental factors, and particularly of dietary factors, in the genesis of the cancer. Among many such studies, those carried out in Manitoba, Canada, on the ethnic distribution of cancer of the gastrointestinal tract (Table 1; Choi, 1968) and on changes in dietary habits between generations of Icelandic migrants in Manitoba (Choi *et al.*, 1971) suggest the importance of certain ethnic foods, such as smoked, pickled and cured meats and fish, in the genesis of gastric cancer (Table 2). These foods are known to contain carcinogenic agents such as aromatic hydrocarbons and *N*-nitroso compounds.

It has been hypothesized that dietary nitrate and nitrite are converted in the stomach to nitrous acid, which then reacts with secondary amines and amides to form nitrosamines and nitrosamides. These compounds have frequently been demonstrated to be carcinogenic in animals (Sander *et al.*, 1975; Mirvish, 1983). Water, especially well-water, may also contain nitrates (National Research Council, 1981). In unrefrigerated food, bacteria can convert

Table 1. Standardized mortality ratios with numbers of deaths from stomach cancer according to ethnic origin by foreign-born and native-born by sex, Manitoba residents, 1956-1965^a

| Ethnic origin | | Foreign born | | Native born | |
|---------------|--------|-----------------|------------------|-----------------|------------------|
| | | Observed deaths | SMR ^b | Observed deaths | SMR ^b |
| Scandinavian | Male | 53 | 248 (185-326) | 29 | 178 (119-256) |
| | Female | 21 | 282 (175-431) | 11 | 135 (67-242) |
| Icelandic | Male | 23 | 391 (248-587) | 16 | 186 (106-301) |
| | Female | 10 | 316 (152-581) | 7 | 166 (66-342) |

^a From Choi (1968)^b In parentheses, 95% confidence limits**Table 2. Groups of foods eaten more frequently by Iceland-born residents of Manitoba, aged 65 and over (based on a score computed for each individual^a) ($\alpha = 0.05$)^b**

| Group of foods | Icelandic (score) | | | | | | Non-Icelandic (score) | | | | | |
|--------------------------|-------------------|------|----|------|----|------|-----------------------|------|----|------|----|------|
| | 1 | 2 | | > 2 | | | 1 | 2 | | > 2 | | |
| <i>Skyr</i> | 30 | 27.3 | 29 | 26.4 | 51 | 46.4 | 101 | 53.4 | 50 | 26.5 | 38 | 28.1 |
| Salted and pickled meats | 30 | 27.3 | 49 | 44.5 | 31 | 28.2 | 96 | 50.8 | 58 | 30.7 | 35 | 18.5 |
| Soured meats | 53 | 48.2 | 24 | 21.8 | 33 | 30.0 | 152 | 80.4 | 22 | 11.6 | 15 | 7.9 |
| Smoked and singed foods | 27 | 24.5 | 36 | 32.7 | 47 | 42.7 | 87 | 46.0 | 63 | 33.3 | 39 | 20.6 |

^a Each food group score for an individual was obtained by adding together the individual's frequency of eating categories, before and after the Second World War, of all foods in the group and dividing by an integer (Categories are numbered 0, 1, 2, 3, for never, seldom, often and very often, respectively.)^b From Choi *et al.* (1971)

nitrites (Weisburger & Raineri, 1975). Preformed nitrosamines have been found in some foods, but most are formed in the stomach, depending on stomach pH and other factors (National Academy of Sciences, 1981).

The human diet appears to include inhibitors as well as promoters of carcinogenesis: vitamins C and E have been shown to inhibit *N*-nitroso product formation by chemically reducing nitrous acid (Mirvish, 1983). A case-control study of diet and gastric cancer was designed to test these hypotheses of cancer promotion and inhibition and to determine the relative risk of ingesting particular food constituents.

Methods

A multicentre collaborative case-control study was conducted during 1979-1982 in Toronto (Ontario), Winnipeg (Manitoba) and St John's (Newfoundland), Canada. Individuals between 35 and 79 years old, newly diagnosed with gastric cancer, were identified in Manitoba and Newfoundland through the provincial tumour registries and in Toronto, through periodic examination of surgical, pathology and medical records in those area hospitals where stomach cancer cases are treated. Of the 565 eligible patients, 250 (44%) were interviewed after initial contact through their physicians. Four cases were excluded owing to unreliable interviews. Controls, identified through door-to-door searches, electoral lists, street directories and municipal enumeration lists, were individually matched to cases for age, sex and area of residence. Of the eligible controls, 58% (250 of 429) agreed to be interviewed; four were excluded, corresponding to the excluded cases. All interviews utilized a standardized, quantitative dietary history questionnaire (Morgan *et al.*, 1978; Jain *et al.*, 1980) and a personal and medical history questionnaire. For the cases, a histology form was completed from hospital records. Then, daily nutrient consumption values were calculated through use of the US Department of Agriculture (1972) Food Composition Data Bank, which was extended and modified for Canadian items (Arthur, 1972; Panalaks *et al.*, 1973, 1974; Gray *et al.*, 1979; McLaughlin & Weihrauch, 1979; National Academy of Sciences, 1981). Continuous conditional logistic regression methods for matched studies (Breslow & Day, 1980) were used for the data analysis to account for the simultaneous and possibly confounding multiple exposures.

Results and discussion

Risk increased with increasing average daily consumption of nitrite-rich foods and decreased with increasing consumption of nitrates, vitamin C and citrus fruits (Table 3), while *N*-nitrosodimethylamine and vitamin E did not appear to affect risk. An analysis of simultaneous consumption of nitrate and ascorbate in 21 common vegetables (Table 4) shows an increased risk for nitrate, instead of the protective effect seen in Table 3, and a more significant reduction in risk for vitamin C. In Tables 3 and 4, each dietary factor was analysed separately in a model which also included total food consumption and ethnicity. In a multivariate analysis simultaneously incorporating high-fibre and high-nitrite foods, as well as total food consumption and ethnicity, nitrites, chocolate and carbohydrate were associated with increasing trends in risk (Table 5). When ascorbate and citrus fruits, which appeared to be somewhat protective when analysed separately, were added to the model of Table 5, they had no significant effect on risk. In the multivariate model, only the consumption of dietary fibre was negatively associated with gastric cancer risk (Table 5).

These findings appear to implicate a number of dietary components, including nitrite consumption, in the genesis of gastric cancer in humans. However, some limitations must be noted. Only 44% of eligible cases participated in the study. Many cases may have been excluded because of early death or severe disease; our findings may represent individuals who were diagnosed at earlier stages or with less severe forms of disease. In addition, gastric cancer patients, as opposed to controls, may have overreported dietary information. It was for this reason that total food consumption was included in all the regression models. We were not able to group case responses by histological type or physical location. Ethnicity was included in the models to represent genetic or familial exposures, because of the known

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Table 3. Trends in gastric cancer risk with nitrite-related factors^a

| Factor | Odds ratio | Unit | 95% Confidence interval | p value |
|-------------------------|------------|------------|-------------------------|---------|
| Nitrite | 1.71 | 1 mg/day | 1.24-2.37 | 0.00061 |
| Smoked meats | 2.22 | 100 g/day | 1.19-4.15 | 0.0077 |
| Smoked fish | 2.03 | 100 g/day | 0.34-12.2 | NS |
| Nitrate | 0.66 | 100 g/day | 0.54-0.81 | 0.00002 |
| N-Nitrosodimethyl-amine | 0.94 | 10 g/day | 0.14-6.13 | NS |
| Vitamin C | 0.43 | 1 g/day | 0.15-1.20 | 0.099 |
| Citrus fruit | 0.75 | 100 g/day | 0.60-0.92 | 0.0056 |
| Vitamin E | 1.01 | 100 mg/day | 0.89-1.14 | NS |
| No refrigeration | 1.19 | 10 years | 1.04-1.35 | 0.0072 |
| Public water supply | 0.86 | 10 years | 0.76-0.99 | 0.029 |

^aEach model also includes total food consumption and ethnicity (British Isles, Oriental, other). In a preliminary analysis, only English ethnicity significantly reduced risk — odds ratio, 0.50 (0.31-0.80). Oriental (Chinese, Japanese, Korean) ethnicity increased risk — odds ratio, 3.09 (0.97-9.86) — but was not significant in a multiple comparison.

NS, not significant

Table 4. Trends in gastric cancer risk with ascorbate and nitrate^a

| Factor | Odds ratio | Unit (mg/day) | 95% Confidence interval | χ^2 | df ^b | p value |
|-------------------|------------------------------|---------------|------------------------------------|----------|-----------------|---------|
| Ascorbate | 0.149 | 100 | {(0.033-0.58) } {(0.904-3.04) } | 8.05 | 2 | 0.018 |
| Nitrate | 1.63 | 100 | | | | |
| All 21 vegetables | (lack of fit of above model) | | | 15.72 | 19 | NS |

^aEach model also includes total food consumption and ethnicity. The 21 vegetables are lettuce, spinach, endive, radish, Brussels sprout, rutabaga, sweet pepper, artichoke, asparagus, okra, beetroot greens, chard, cucumber, zucchini, cauliflower, broccoli, cabbage, kale, pea, turnip and turnip greens.

^bdf, degrees of freedom

NS, not significant

association of ABO blood types and gastric cancer (Bjelke, 1980). If differing gastric cancer risks for various ethnic groups were due to different typical diets, the ethnic factor would bias the analysis of dietary component risks. However, since ethnicity odds ratio estimates did not change greatly when food components were added to the models, ethnicity appears to represent nondietary factors.

Our results are consistent with those of previous studies of diet and gastric cancer. Increased risk with consumption of smoked meats and fish has been shown in a number of studies (Meinsma, 1964; Higginson, 1966; Haenszel *et al.*, 1972, 1976; Bjelke, 1979; Juha'sz, 1980). Our finding of increased risk with chocolate consumption is supported

Table 5. Trends in gastric cancer risk with calculated consumption of food constituents^a

| Factor | Odds ratio | Unit | Confidence interval | <i>p</i> value |
|----------------------------|------------|-----------|---------------------|-------------------|
| Dietary fibre ^b | 0.40 | 10 g/day | 0.28-0.58 | <10 ⁻⁸ |
| Nitrite | 2.61 | 1 mg/day | 1.61-4.22 | <10 ⁻⁴ |
| Chocolate | 1.84 | 10 g/day | 1.22-2.77 | <10 ⁻⁴ |
| Carbohydrates | 1.53 | 100 g/day | 1.07-2.18 | 0.015 |
| No refrigeration | 1.17 | 10 years | 1.01-1.35 | 0.037 |

^aModel simultaneously includes all factors shown, as well as total food consumption and ethnicity

^bDietary residual after digestion by stomach enzymes, etc
NS, not significant

by the work of Modan *et al.* (1974) and by those of Haenszel *et al.* (1972) and Jedrychowski *et al.* (1980), who observed increased risk with consumption of sweets. The protective effect of citrus fruit seen in our study has been observed by some researchers (Meinsma, 1964; Higginson, 1966; Bjelke, 1979) but not by others (Acheson & Doll, 1964; Graham *et al.*, 1972; Haenszel *et al.*, 1972).

Our results, in agreement with those of previous studies, strongly support the hypotheses that nitrite intake is associated with increased stomach cancer risk and that consumption of citrus fruits appears to be somewhat protective. The strong trends for increased risk with chocolate consumption and decreased risk for fibre consumption suggest that much remains to be elucidated about the complex role of dietary components in carcinogenesis.

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